A PCR based microbial monitoring alternative method of detection and identification of microbes aboard ISS

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Previous research has shown that microorganisms and potential human pathogens have been detected on the International Space Station (ISS) with additional introduction of new microflora occurring with every exchange of crew or addition of equipment and supplies. These microbes are readily transferred between crew and subsystems (i.e. ECLSS, environmental control and life support systems). As this can be detrimental to astronaut health and optimal performance of ISS systems, monitoring of systems such as ECLSS to include identification of microbial contaminants could prevent adverse effects on human health and life support systems.

Current monitoring on ISS is laborious and utilizes culture based methods followed by sample return to Earth for complete analysis. Future, long-distance spaceflight missions will require real-time monitoring capabilities that enable efficient and rapid assessments of the microbial environment allowing for expedited decisions and more targeted response to cope with anomalies.

Polymerase chain reaction (PCR), a molecular microbial monitoring method was chosen and numerous PCR instruments investigated for their potential to perform in microgravity conditions. Using ISS as a test bed for PCR verification in microgravity will enable NASA to assess whether molecular based microbiological sensors may be components of reliable, closed-loop life support and habitation systems in spacecraft, enhancing infrastructure capabilities through increased efficiency, reliability, and time savings by enabling sample analysis on orbit. NASA selected the Water Monitoring Suite as one of the rapid spaceflight hardware demonstration activities utilizing a streamlined process to minimize the time required to fly experimental flight hardware. The RAZOR EX (BioFire Defense, Salt Lake City, UT) system was part of the water monitoring suite and is a commercial off-the-shelf (COTS) real-time PCR instrument designed for field work. The RAZOR EX was originally designed for Department of Defense (DoD) under a small business innovative research (SBIR) grant and is ruggedized, compact and provides a rapid, sample to answer in less than an hour.

PCR assays using a fluorescent probe were optimized and spiked with known concentrations of DNA (*Pseudomonas aeruginosa*) ranging from 0.002 to 20 ng. PCR reagents were lyophilized and configured in customized pouches and tested for flight readiness. Three types of water

were used to rehydrate the reagents and demonstrate the fidelity of the PCR reaction in microgravity. Molecular grade deionized water served as a control while filtered and unfiltered ISS potable water served to test for chemical or biological inhibitors. All three types were compared to parallel ground test results.

Nine tests were run on ISS (3 of each water type) and the critical threshold cycle (Ct) was compared to parallel ground tests completed at Kennedy Space Center, FL and Johnson Space Center, TX. All concentrations of *Pseudomonas aeruginosa* DNA were detected. A comparison of the Ct produced in real time PCR indicated similarity between flight and ground samples. There appeared to be no significant difference between flight or ground PCR reactions or between any of the three water types.

This testing demonstrated the ability to perform molecular testing during spaceflight operations with similar sensitivity. It will allow for future ground development of molecular protocols and minimize the need for spaceflight testing. Future testing will include development of additional targets including environmental and health related organisms.